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# PHYLLOSTICTA LEAF SPOT AND DAMPING OFF OF SNAPDRAGONS

E. F. GUBA AND P. J. ANDERSON

WITH EIGHT FIGURES IN THE TEXT

During the autumn of 1918 a leaf spot disease was prevalent among the cultivated snapdragons (*Antirrhinum majus* L.) in the perennial gardens of the Agricultural College at Amherst, Massachusetts. The spots were much larger and in other ways quite different from those produced by the anthracnose fungus (*Colletotrichum Antirrhini* Stew.). A search through the literature failed to show that a leaf spot of this character had previously been described or reported from America. In order to make further investigations, small "volunteer" plants which had started in the beds

FIG. 1. LEAF LESIONS ON TWO INOCULATED PLANTS

about the old ones were potted and brought into the greenhouse. They were watered frequently at first in order to get them well established in the pots, but after a week or two a number of them had damped-off and were lost. Examination of the damping-off lesions showed that this was only another symptom of the disease which had been found on the leaves. This led the writers to examine again the out of door plants and, although it was late in the season and the plants were frozen, stem lesions were discovered. They were usually connected with dead leaves, indicating that the disease had resulted from leaf or petiole lesions. Since the disease

appeared to be of some importance and is probably prevalent in other sections, the results of further studies are presented here.

*Symptoms.* On the leaves the disease manifests itself as large circular dark brown or black spots usually showing a series of concentric ridges which produce a target-board effect much like the lesions of the early blight of potato. The spots are typically about 1 cm. in diameter and

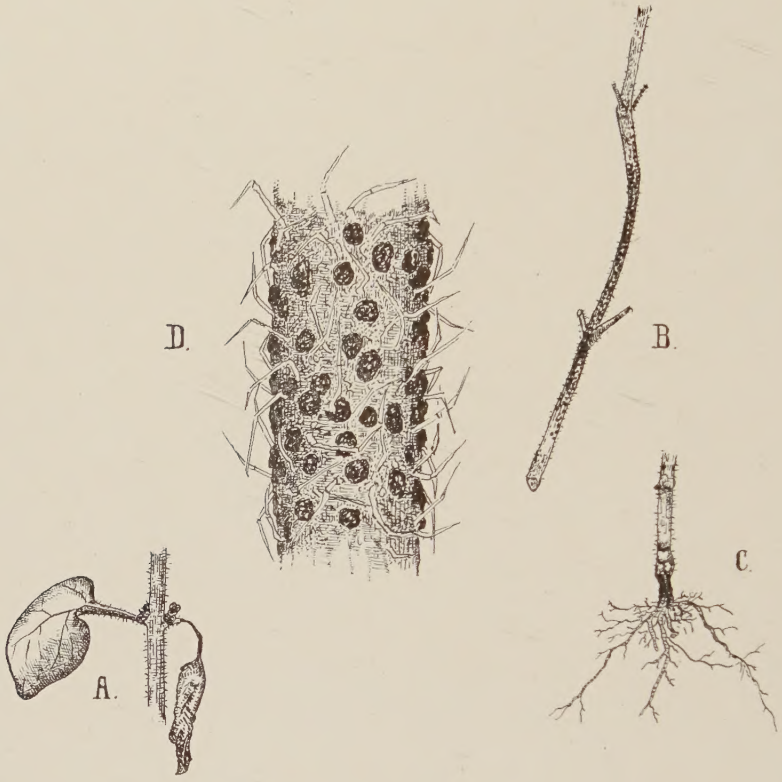


FIG. 2. *a*, PETIOLE LESION CAUSING DEATH OF THE LEAF; *b*, STEM LESION SHOWING CONSTRICTION: *c*, DAMPING-OFF LESION: *d*, ENLARGED SECTION OF A STEM LESION SHOWING PYCNIDIA

usually only one or two on a leaf but sometimes as many as five have been observed. When more than one spot occurs on a leaf, they usually merge and form large irregular blotches. They may be located on any part but show a preference for the tips and edges. Lesions on the edge of the leaf are at first elongated but later advance inward and become semi-circular. Under favorable conditions of moisture, the leaf spots do not

appear to be determinate but continue to spread until the entire leaf is involved.

The spots begin as small yellow unglazed areas as distinguished from the glossy green of the healthy tissue, accompanied by shrivelling and depression. The center of the spot, or starting point of infection, gradually becomes yellow-brown and transparent, revealing the venation of the leaf when wet. The yellow-brown then alternates outwardly with concentric rings of darker brown. A hand lens shows numerous tiny dark points associated with the darker rings or scattered sometimes irregularly over the surface of the spot. Leaves attacked when young become distorted

FIG. 3. PLANT AFFECTED BY DAMPING-OFF COMPARED WITH A HEALTHY ONE

and curled (fig. 1), and if the attack is severe the whole leaf shrivels and dies but remains clinging to the stem. Lesions become dry, hard and brittle, readily breaking to pieces when handled.

Brown elongated lesions occur on the petioles. The petiole is quickly girdled and the leaf droops and dies (fig. 2, a). Almost invariably also the petiole lesions spread downward and result in stem infections at the axils of the leaves.

The stem lesions are dark green or brown, without definite margins and may extend along one side for 2 or 3 cm. before the stem is girdled. The lesions then become dark and constricted and dotted over with numerous black points like those on the leaves (fig. 2, *b* and *d*). The stem usually

bends over at the point of attack. Soon all parts above die and become brittle, hard and dry.

The first noticeable symptom of damping-off is the rather rapid wilting and dying of all parts above ground (fig. 3). Examination of the base of the stem reveals the characteristic brown-black lesion considerably constricted and beset with the same numerous black specks (fig. 2, *c*).

*Isolation.* Microscopic examination showed the small black specks scattered over the lesions to be pycnidia. Isolations were made by transferring small bits of the diseased tissue to potato agar slants after the surface of the lesion had been sterilized with mercuric chloride (1–1000) and washed with sterile water. Some difficulty was at first encountered in

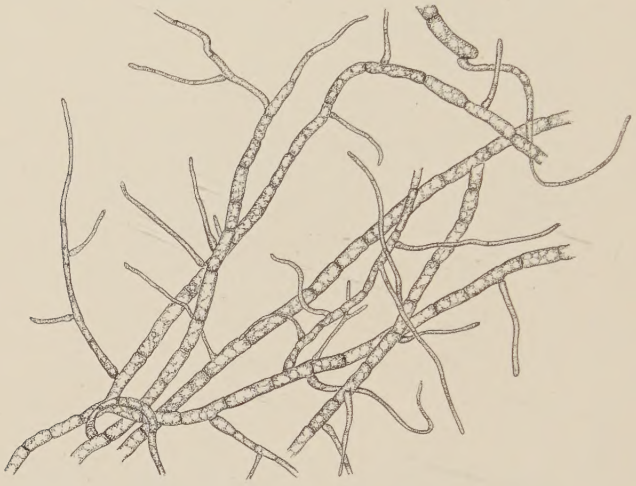

FIG. 4. MYCELIUM FROM CULTURE

securing the pycnidial form in pure culture because of an Alternaria which was usually present on the lesions and which grew in culture more rapidly than the other fungus. The Alternaria was also kept in pure culture with the expectation of testing out its pathogenicity, but since—as is shown below—the pycnidial form alone was able to produce the disease, it was concluded that the Alternaria was secondary and no further attention was given it.

At an incubating temperature of 28°C. growth becomes perceptible on the agar within twenty-four hours. At the end of four days one half of the surface of the slant is covered with a rather thin, flat growth and pycnidia are visible. They are most numerous about the center of the growth but never so thick as to give it a solid black appearance. They are at first of a yellow brown color but become dark with age. As the

culture becomes older, radiating streaks of dark brown develop, extending outward toward the periphery. Sometimes the whole culture takes on this dark color. The pycnidia develop most numerously along these dark streaks. The characters and measurements of pycnidia and spores in culture were found to be identical with those of the fungus on the leaves.

*Mycelium.* The characters of the mycelium in culture and in the host appear to be the same. The black streaks in the cultures at first give one the impression of a mixed culture, but microscopic examination and further culturing prove that such is not the explanation. The hyphae are at first hyaline but in the streaks they become brown and the walls are thicker. The thallus is composed of large stout main branches which may or may not take on the brown color, and much smaller lateral branches which diverge at acute or right angles from the stouter ones (fig. 4). The stout hyphae

FIG. 5. ENLARGED LEAF SPOT SHOWING PYCNIDIA

are rather closely septate forming cells $1\frac{1}{2}$ to 2 times as long as broad and are constricted at the septa. The lateral branches are hyaline, more distantly septate and not constricted at the septa. In the host the mycelium is intercellular.

*Pycnidia.* The pycnidia develop directly under the epidermis. With age the epidermis is pushed up and is finally ruptured by the ostiole. The ostiolate end of the pycnidia may emerge from either side of the lesion: rarely, however, from the lower side. The pycnidia are very prominent, and distinctly visible from the upper side (fig. 5). They can also be seen from the lower side due to the transparency of the lesion which occurs quite frequently, especially when the lesions are wet. They show no differences in culture and on the host. They are globose and range in diameter from 78 to 152$\mu$ with an average of 108$\mu$. The ostiole is papilliform and round. The wall is at first light yellow brown but becomes

darker with age. The wall of the pycnidium is very thin, consisting usually of only two layers of thin tabular cells (fig. 6). The cell walls of the outer layer are thicker and impart the dark color to the pycnidium. Seen in face view they are irregularly hexagonal and of a diameter of 10 to 12$\mu$ (fig. 7). The conidiophores have not been seen. If present they must be very short and inconspicuous. The interior of the pycnidium is densely packed with spores which issue from the ostiole in long gelatinous cirrhi. When the pycnidia are mounted in water, these rope-like strands may be watched for twenty minutes or more pushing out of the ostiole and remaining for a long time in regular formation (fig. 7).

*Spores.* The spores are continuous, hyaline, oblong or oval to elongate, straight, with homogeneous contents. Spores from the host measured

FIG. 6. LONGISECTION OF A PYCNIDIUM

3.1 to 4.27 by 1.5 to 2.1 (average 3.8 by 1.9$\mu$). In culture they averaged 4.2 by 2$\mu$ (fig. 8, *a*).

*Germination of spores.* Immersed in a drop of water in a Petri dish and kept at ordinary laboratory temperature, the spores germinate in sixteen to thirty hours. Germination is accompanied by a great increase in size of the spores, the average after thirty-six hours being 10 by 7$\mu$. One, two, or three germ tubes may arise from a single spore but the usual number is two (fig. 8, *b*). The tubes are commonly polar. They quickly become septate and branched. Anastomosing of the branches from the same spore or from different spores is very common. Germination seemed to be best at about 25°C. but no extensive determinations were made of the optimum, maximum and minimum. They are not killed by exposure to winter temperatures. Pycnospores from plants which had been out

of doors all winter were used for infection experiments during February and produced the disease as readily as spores from fresh cultures.

*Infection experiments.* Healthy plants which had been growing for two months in the greenhouse were used for infection experiments. Pycnidia from pure culture were crushed in sterile water and the spore suspension thus obtained was sprayed over the plants with an atomizer. The sprayed plants were covered for several days with bell jars. Throughout the entire set of experiments, the lesions seldom became visible before nine days but developed very rapidly after they started. In order to see whether wounds are necessary, the leaves of two plants were punctured

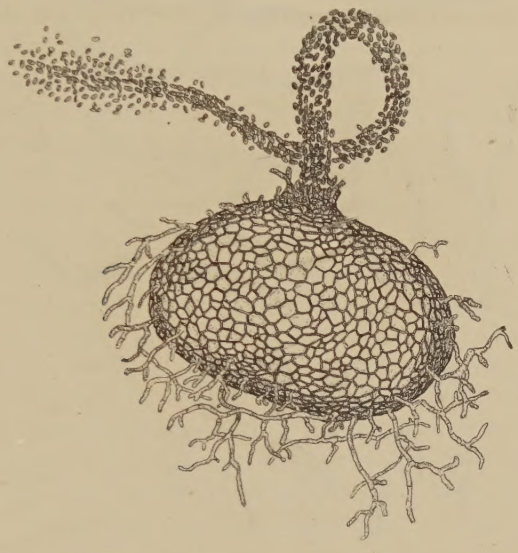

FIG. 7. DETACHED PYCNIDIUM WITH EXUDING SPORES

by numerous stabs of a sterile needle. Two others were not injured and all four were sprayed with spores at the same time and kept under the same conditions. Lesions became apparent on the stabbed leaves within five days. On the uninjured leaves they were not seen until the ninth day. Also they were not so numerous as on the stabbed leaves. Wounds then do not seem to be necessary for infection but, on the other hand, they do furnish good infection courts. Since they were not necessary, none of the plants in numerous subsequent experiments were wounded but infections invariably resulted. In another set of experiments, bits of agar from pure cultures containing mycelium and spores were applied to the leaves without wounding them. Lesions always became apparent about the points of application within nine days while the checks remained healthy.

Pycnidia begin to appear on the fourth or fifth day after the lesion appears, first a few on the yellow-brown center of the spot and then later more numerously on the concentric darker zones but in old spots they may be scattered irregularly over the entire surface.

The effect of moisture conditions of atmosphere and soil is very marked. When the plants are kept continuously in a moist atmosphere such as may be secured by placing them in an enclosed chamber, the disease continues to spread until eventually the entire plant is killed. But, if the atmosphere is dry and the plants are not heavily watered, the lesions may cease

FIG. 8. *a*, PYCNOSPORES; *b*, GERMINATING PYCNOSPORES

to spread altogether or grow only very slowly. The damping-off type is especially favored when the soil is kept in a very moist condition.

*Reisolation.* Isolations were made from the spots which had been produced by inoculation of leaves and stems. A fungus was secured in pure culture which in all characters was like the one which had originally been isolated from the plants in the perennial gardens. The pathogenicity of this fungus has thus been established by (1) finding it constantly associated with the disease, (2) isolating and studying it in pure culture, (3) producing the disease on previously healthy plants by inoculating with spores from pure culture while the checks remained healthy and (4) reisolating the fungus from the inoculated plants and proving from morphological

and cultural characters that it was identical with the fungus originally isolated.

*Identity of the causal fungus.* The globose, ostiolate, dark-colored, thin-walled pycnidia situated beneath the epidermis in definite leaf spots, and the ovate or elongate, continuous, hyaline spores locate this species at once in the genus Phyllosticta. Turning to the literature we find that only one species of Phyllosticta has been reported on this host, *Phyllosticta Antirrhini* Syd., which was described[1] from plants in Berlin, Germany, as follows:

"*Phyllosticta Antirrhini* sp. n. Maculis amphigenis, in epiphyllo pallescentibus, in hypophyllo virescentibus, plerumque marginalibus, v. apicalibus, usque 1 cm. longis, definitis, interdum subzonatis; peritheciis epiphyllis, minutis, globosis, ca. 100–180$\mu$ diam., sparsis v. subgregariis, atrobruneis; sporulis oblongis, continuis, hyalinis, eguttulatis, 4–6 x 1½–2.

"Hab. in foliis Antirrhini spec., pr. Berolinum, Germaniae."

No other reference to the leaf-inhabiting fungus has been found by the writers in European or American literature. No European material has been examined but the description and measurements correspond rather closely with those of the fungus as found here. There appears to be no good reason for believing the two to be distinct.

When, however, the same fungus was found on the stems, the possibility was suggested that it had been described as a Phoma. The artificial basis of classification, by which all such fungi as this inhabiting leaf spots are called Phyllosticta while those on other parts of the plant are called Phoma, does not offer a satisfactory method of classifying those which occur on both stem and leaf. A Phoma stem rot of snapdragons was described by Stewart[2] from New York in 1900. He found that this fungus girdled the succulent stems and caused the tips to die. He isolated it and was able to produce the disease by inoculation. Spore measurements are given as 4 to 5 by 2$\mu$ and it is stated that the spores issue from the ostiolum in colorless, gelatinous, rope-like masses. No specific name was given to this Phoma. Professor Stewart kindly furnished dried specimens for the writers to examine and they were unable to find any essential difference between it and the Phyllosticta under investigation. Professor Stewart also is of the opinion[3] that the two may be identical.

In short then it seems that the large leaf spot, the damping-off and the stem rot are caused by the same fungus and may be considered as

[1] Sydow, P. Beitrage zur Kenntniss der Pilzflora der Mark Brandenburg II. Beitblatt zur Hedwigia **38**: (134). 1899. Same in Sacc. Syll. **16**: 839. 1902.

[2] Stewart, F. C. An anthracnose and stem rot of the cultivated snapdragon. N. Y. (Geneva) Agr. Exp. Sta. Bul. 179. 1900.

[3] Private correspondence with the senior writer.

only different symptoms of the same disease. Since the organism was originally described as a Phyllosticta and does cause a definite leaf spot, it will undoubtedly be best to retain the name *Phyllosticta Antirrhini* Syd. even though it does infest the stem also.

*Control.* Stewart suggests spraying the plants with Bordeaux mixture as for anthracnose but his suggestion was apparently not based on experiments with this particular fungus. The efficiency of a few common fungicides was determined by the present writers in the laboratory. By means of an atomizer, glass slides were sprayed with (a) Bordeaux mixture 4–4–50, (b) ammoniacal copper carbonate, (c) lime-sulphur 1–30, 1–40 and 1–50 and permitted to dry for one or more days. A drop of water containing fresh spores of the fungus was placed on the center of the sprayed surface of each slide and the slides kept in Petri dishes to prevent evaporation of the water. Similarly, drops of the spore suspension were placed on unsprayed slides for checks. Percentages of germination were counted after seventy-two hours. The average results of five series are as follows:

| *Fungicide used* | *Percentage of germination* |
|---|---|
| Lime–Sulphur, (1–30) | 27 |
| Lime–Sulphur (1–40) | 27 |
| Lime-sulpuhr (1–50) | 22 |
| Bordeaux mixture | 0 |
| Ammoniacal copper carbonate | 37 |
| Checks | 86 |

Results with the lime-sulphur were puzzling and not constant, but indicate without question that this fungicide is not efficient at the strengths tested. Bordeaux mixture alone gave satisfactory results and was, therefore, employed in the control experiments on the plants in the green house.

On February 5 four plants were inoculated with a spore suspension in the manner already described. Three of these plants were sprayed with standard Bordeaux mixture previous to inoculation. Lesions began to appear on the unsprayed plants on the ninth day after inoculation and by March 6 most of the leaves were affected and many of them dead. Plants sprayed with Bordeaux showed no lesions whatever.

On February 19th, five more plants were inoculated, three of which were previously sprayed with Bordeaux mixture. By March 7, 50 per cent of the leaves of the unsprayed plants were either dead or badly affected while those of the sprayed plants were perfectly healthy. One of the sprayed plants, however, succumbed to a damping off lesion on March 5.

These experiments indicate that all types of this disease with the exception of damping-off can be controlled by spraying with Bordeaux

mixture 4–4–50. In the case of damping-off the fungus enters the plant at a point below the surface of the soil and spraying is obviously not to be depended on. It seems likely that the pathogen lives in the soil. If damping-off should prove to be serious, soil sterilization of the seed bed with formaldehyde or steam should be efficient. Succulent shoots are very susceptible to the fungus whereas the woody stems are not so easily affected. In checking the disease it is very essential that the air and soil be kept as dry as is consistant with good growing conditions for the plants.

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